# STUDIES ON THE CHARACTERISATION OF BIOMARKERS OF NUTRITIONALLY-DERIVED STRESS IN PARALARVAL CULTURES OF THE COMMON OCTOPUS (*Octopus vulgaris*)

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## Introduction

Nowadays, due to the high mortality within the first 30 days of life, octopus paralarvae culture represents the main obstacle for commercial production of this species. The causes of such mortality are not yet well defined and understood. As a part of a broader project aimed at characterising the causes of such massive mortality, we envisaged the study of nutritionally-derived stress, through the selection of biomarkers capable of its detection and quantification.

## Materials and Methods

Paralarval cultures starved and fed *Nannochloropsis* sp - and *Isochrysis galbana* - enriched *Artemia*, and *Artemia* plus crustacean zoeae (*Maja* sp.), were raised up in 500 l tanks until the outburst of massive mortalities. Two experiments were carried out that lasted 14 and 30 days respectively. Samples were taken at 0, 5 and 14 days in experiment 1 and at 0, 4, 16 and 30 days in experiment 2. The following biomarkers were analysed: RNA/DNA, stress protein Hsp70, supeoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), Se-dependent glutathione peroxidase (Se-GPX), the isozymic pattern of SOD, and the levels of malondialdehyde (MDA). Essays related with antioxidant defenses were performed only in samples from experiment 2 at hatching and at days 16 and 30 due to problems of availability.

RNA and DNA were quantified following the procedure described in Varó et al. (2007), using RiboGreen ™ RNA Quantitation Kit and PicoGreen ™ DNA Quantitation Kit respectively (Molecular Probes). HSP70 were separated by 1D-SDS-PAGE using a Mini-Protean Tetra cell system (Bio-Rad), and transferred onto PVDF membranes in a Trans-Blot$^{R}$ Turbo Blotting system (Bio-Rad). Blots

were visualized on a VERSADOC Imaging system (Bio-Rad) using ELC-PRIME reagent (Amersham), and quantified by densitometry. Specific activities of antioxidative enzymes and MDA levels were determined according to Pérez-Jiménez et al. (2009), as well as SOD isoforms, determined by PAGE (MiniProtean II, Bio-Rad). CuZn-SODs were inhibited by CNK (50 mM) and types of SOD were detected by the photochemical nitroblue tetrazolium (NBT) staining method. Data were compared with ANOVA followed by Tukey's test (more than 2 groups), and with Student t-tests (Significant differences: $p<0.05$).

**Results and discussion**

After hatching, 4 to 5 days of starvation show up in a decrease in the values of RNA/DNA ratio and HSP70 as a consequence of nutritional stress (Table I). From days 4 to 30 the RNA/DNA remained unchanged irrespective of diet. The levels of HSP70 were higher in the dietary groups, and especially higher in the paralarvae fed *Artemia* up to 5 days. At the end of experiment 1(14 days) lower values were found probably as consequence of mortality. Paralarvae fed zoeae in the second experiment, showed higher HSP70 values, possibly reflecting a better nutritional status that correlated with higher growth (data not shown), and pointing at this biomarker as a sensitive indicator.

Enzymatic results show that paralarvae possess a complete enzymatic pool, with high activity (especially Mn-SOD) as compared to other cephalopods (Table II). There is an increase in CAT and Se-GPX activity during development. There is not oxidative damage associated to the feeding regimes, probably due to the antioxidant properties of the diets. Besides, there are not clear changes in enzymatic activities associated to growth or survival, except Se-GPX activities that were higher in the paralarvae fed zoeae than in those fed *Artemia*; in agreement with a better growth and survival and reflecting the differences in such enzyme activities between live preys. Also, the SOD isozymic profiles reflect those of live preys.

**Conclusions**

The results point at the RNA/DNA ratio as an indicator of starvation, and at the levels of HSP70 and Se-GPX activity as more sensitive biomarkers of the nutritional status of paralarvae. There is not evidence of a prooxidative status promoted by diets, but a detailed analysis of oxidative damage to proteins could help to associate oxidative damage to mortalities and growth.

**Acknowledgements**

This research was supported by "Ministerio de Ciencia e Innovación" through

the OCTOPHYS Project (AGL-2010-22120-C03-02). OM was supported by a Marie Curie R.G. (PERG08-GA-2010-276916, LONGFA) and by a Juan de la Cierva postdoctoral contract. The study was also carried out under the scope of a PROMETEO Project (2010/006) from Generalitat Valenciana.

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